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14. ABSTRACT

Sam68 is a target for *Src* kinase. It is involved in HIV-1 RNA export. However, the role of Sam68 in cellular mRNA export is unknown. It has been well documented that patients have either lost or have mutations in BRCA1 or BRCA2 tumor suppressor genes are at high risk of developing breast cancers. Thus, the goal of this application is to investigate whether Sam68 is involved in the export of BRCA1 mRNA. To test this objective, we have generated stable Sam68 knockdown MCF7 cells. These reagents are critical to objectively test the role of Sam68 on BRCA1 expression. In this no-cost extension year, using MCF7 Sam68-RNAi stable cells, we expect to be able to examine how the reduced expression of Sam68 affects BRCA1 mRNA export. We will also identify the RNA export pathway for BRCA1 mRNA, proposed in Aim 2.

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Breast Cancer

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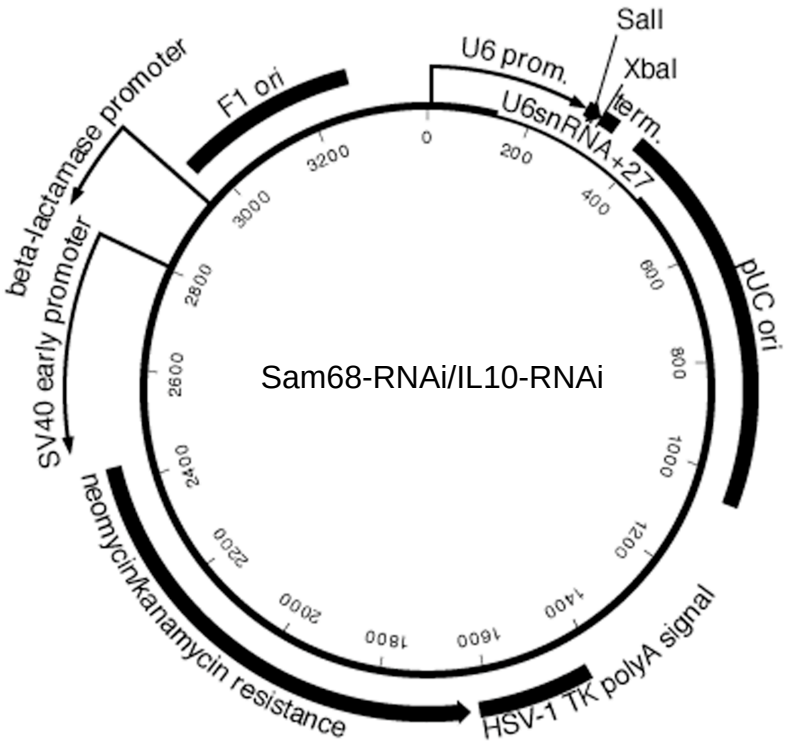
Construction of Sam68-RNAi vector:

We used SuppressorNeo-IMG-800 (Imgenex Corp., San Diego, CA) expression vector to generate the stable Sam68 and IL10 knockdown MCF7 cells. Following primers were annealed and cloned into the *Sal* I and *Xba* I restriction enzyme sites of pIMG-800 to generate pSam68-RNAi and pIL10-RNAi using standard protocol.

Sam68 (forward primer: 5'-TCGAGGGATGATGAGGAGAATTACGAGTACTGGTAA-TTCTCCTCATCATCCTT TTT-3' and reverse primer: 5'-CTAGAAAAAGGATGATG-AGGAGAATTACCAGTAC TCGTAATTCTCCTCATCATCCC-3').

IL10 (forward primer: 5'-TCGAGCATACT GCTAACCGACTCCGAGTACTGGGAGT-CGGTTAGCAGTATGTTTTT-3' and reverse primer: 5'-CTAGAAAAACATACTGCA-ACCGACTCCCAGTAC TCGGAGTCGGTT AGCAGTATGC-3').

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SAM68-RNAi                          IL10-RNAi

5'-GGATGATGAGGAGAATTAC-3'       5'-CATACTGCTAACCGACTCC-3'
3'-CCTACTACTCCTCTTAATG-5'       3'-GTATGACGATTGGCTGAGG-5'
```

Figure 1: Schematic diagram of RNAi expression vector (IMG-800), and Sam68-RNAi and IL10-RNAi target sequences.

Creation of stable Sam68 knockdown MCF7 cells:

The main objective of this study was to deplete intracellular Sam68 and determine if a reduced expression of Sam68 would impact BRCA1 RNA export. To produce MCF7 cell lines with constitutively reduced levels of Sam68 expression, we have transfected MCF cells with pSam68-RNAi or pIL10-RNAi and placed under G418 selection. Sam68 expression in G418 resistant clones was analyzed by Western blot analysis using anti-Sam68 antibodies (Fig. 2). Out of 5 clones analyzed, two of them (#2 and 5) had reduced amounts of Sam68. Expression of endogenous Sam68 in these clones was reduced to 20-40% of the level in MCF7-IL10i cells (#6 and 7) as assessed by

immunoblot analysis (Fig. 2). The successful establishment of Sam68 knockdown MCF7 cell lines demonstrates that reduced levels of Sam68 were not toxic.

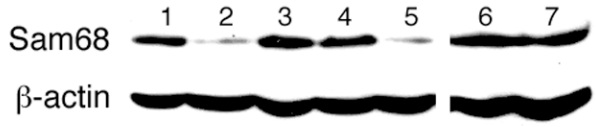

Fig. 2: Stable knockdown of Sam68 in MCF7 cells: Expression levels of Sam68 in stable clones were assessed by Western analysis using anti-Sam68 antibodies. Stable Sam68 knockdown MCF7 cells (clones #1 to 5). MCF-IL10i (clone #6 and 7); β-actin was used as internal control.

In this no-cost extension year, using MCF7 Sam68-RNAi stable cells, we expect to be able to determine the effect of Sam68 knockdown on BRCA1 expression. We will also identify the RNA export pathway for BRCA1 mRNA, proposed in Aim 2. There is no change from our original objectives.

We also would like to point out that this grant (BR046108) has been acknowledged in the following publication:

1. Badri K*., Suhasini M*., Gerard H., Khan I, Bagchi M, Hudson AP., and Reddy TR. Regulation of Sam68 activity by small heat shock protein 22. J. Cellular Biochem. 99:1353-62 (2006) * equally contributed